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GENERAL DIRECTOR

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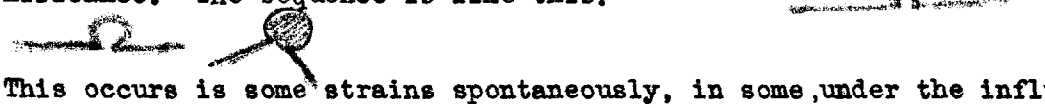
(CONVALESCENTS)

May 12, 1958

Dear Dr. Lederberg:

I read with great interest the manuscript on protoplasts and L forms which you sent me some time ago and which has since appeared in the Journal of Bacteriology. I wanted to write you immediately but was prevented by various things and then I hope to see you at the meeting in Chicago. I would like to talk over certain aspects of these problems with you and I hope you will visit us sometime in Boston. Meanwhile I would like to make a few remarks on your observations.

The attempt to use L forms for genetic studies was not very fruitful but it was a great pleasure for me personally that some of the experiments which I have for a long time wished to make were made. However, it is possible that this is not the last word on this problem. There are certain phenomena which suggest that the formation of large bodies in some cases may have genetic significance. I have repeatedly referred to it that in some strains of typhoid and colon bacillus the large bodies are formed by fusion and it is difficult to believe that this process is without significance. The sequence is like this:



This occurs is some strains spontaneously, in some, under the influence of penicillin. The observations with diaminopimelic acid are of great interest and I look forward to seeing someone undertake an extensive biochemical study of the various L forms. I follow Dr. Sharp's work closely but I do not feel fit to go into it myself. The large bodies which are the precursors of L forms are produced by various influences such as exposure to cold and to chemicals as variable as amino acids and heavy metal salts. It is unlikely that the biochemical processes are similar in all these cases. Apparently bacteria are able to continue growth when they remain huddled together without division in conditions in which growth of single individuals is impossible. A high degree of osmotic protection is not necessary in most cases for the growth of the large bodies. Under certain conditions the elements which produce the large body by growth are able to grow alone outside of the original large body. I do not believe that the use of the term 'protoplast' is really appropriate either for the large bodies or for the growing elements inside them.

This term is used mainly for protoplasts induced by lysozyme. These are produced under highly artificial conditions by direct injury to the cell wall and they do not multiply in this form. ~~xxx~~ In contrast, the large bodies are produced by acvive growth and reproduction. It was apparent in several cases that they contain preformed 5 or more elements which under appropriate conditions will develop into bacteria as well as multiply in such form as they are in the large bodies. They do not have the usual cell wall but in morphology and potential development are quite different from the forms produced by lysozyme. The process by which you explain the growth of L forms from the large bodies does not, according to my experience, have general significance. Granules grow into the agar from the surface without being exposed to any pressure and they grow to a certain extent on coagulated serum into which they do not penetrate. The size of the granules varies greatly but in young colonies is often not more than 0.3 to 0.5 μ, and if they are properly stained they have as distinct morphology as the bacteria. My impression is that the basic morphology is a very small bipolar rod-like structure which rounds up and swells in the same way as often occurs with Pasteurella and H. influenzae. The morphology is strikingly similar to that of the pleuropneumonia group and also to the morphology of the large viruses. At the recent meeting in Chicago I had the opportunity to see micrographs of thin sections of cells infected with psittacosis. I received last summer a micrograph from Weibull from thin sections of Proteus L forms. The structures in both micrographs were quite indistinguishable,- thin-walled vesicles containing oval granules of about 0.2 μ. These similarities suggest that sometimes in the past the L forms played a role in adaptation to parasitic life. Whether this happens ~~to~~ at present, an open question.

It seems to me quite important that L forms with different properties can often be obtained from a strain of bacterium. The differences between the forms which I call 3A and 3B are clear cut. All your observations were made with 3B strains and agree with our observations made with such strains. Some strains of E. coli produce large bodies on the usual media and slight L growth corresponding to the 3A type. The use of high concentrations of salt and sucrose makes it possible to grow some L forms and we succeeded recently to grow L forms from the pneumococcus with sucrose. However, in many other cases in which large bodies are produced without difficulty, but not L forms, osmotic protection gave no help , e.g., in Neisseria and Pasteurella.

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The impression that the L forms represent the growth of the inside of bacteria without producing the usual cell wall was told to me by Dr. Oerskov as early as 1936. In the case of Proteus it was apparent that the content of a filament can herniate out in distilled water forming a large body which when transferred to nutrient agar reproduces either bacteria or L forms. This seems to me even today the most clear-cut observation indicating that bacteria can survive without the cell wall. The remarks which I made in the Review on the nature of L forms started from this observation. However, I am not sure that this solves the whole problem. If the morphology of the L forms is such as I indicated above, they might have some kind of an envelope, especially the tiny rod-like forms.

I hope that you will continue to be interested in these peculiar forms. It arouses new interest to see a fresh approach different in some respects from mine.

With kind regards,

Sincerely yours,

Louis Dienes